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Blockade of adenosine A_1 receptors prevents methylphenidate-induced impairment of object recognition task in adult mice

Sabrina Mioranzza [a], Marcelo S. Costa [a], Paulo Henrique S. Botton [a], Ana Paula Ardais [a], Vanessa Lague Matte [a], Janaina Espinosa [a,b], Diogo O. Souza [a], Lisiane O. Porciúncula [a,b,*]

[a] Federal University of Rio Grande do Sul, Health and Basic Sciences Institute, Department of Biochemistry, Graduation Program in Biological Sciences — Biochemistry, Laboratory of Studies on the Purinergic System, Porto Alegre/RS, Brazil 90035-003
[b] Graduation Program in Biological Sciences — Neurosciences, Porto Alegre/RS, Brazil 90035-003

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ABSTRACT

Methylphenidate (MPH) is the preferred treatment used for attention-deficit/hyperactivity disorder (ADHD). Recently, misuse for MPH due to its apparent cognitive enhancer properties has been reported. Adenosine is a neuromodulator known to exert influence on the dopaminergic neurotransmission, which is the main pharmacological target of MPH. We have reported that an overdosage of MPH up-regulates adenosine A_1 receptors in the frontal cortex, but this receptor was not involved in its anxiolytic effects. In this study, the role of adenosine A_1 receptor was investigated on MPH-induced effects on aversive and recognition memory in adult mice. Adult mice received acute and chronic (15 days) administration of methylphenidate (5 mg/kg, i.p.), or an acute overdosage (50 mg/kg, i.p) in order to model misuse. Memory was assessed in the inhibitory avoidance and object recognition task. Acute administration 5 mg/kg improved whereas 50 mg/kg disrupted recognition memory and decreased performance in the inhibitory avoidance task. Chronic administration did not cause any effect on memory, but decreased adenosine A_1 receptors immunocontent in the frontal cortex. The selective adenosine A_1 receptor antagonist, (DPCPX 1 mg/kg, i.p.), prevented methylphenidate-triggered recognition memory impairment. Our findings showed that recognition memory rather than aversive memory was differently affected by acute administration at both doses. Memory recognition was fully impaired by the overdosage, suggesting that misuse can be harmful for cognitive functions. The adenosinergic system via A_1 receptors may play a role in the methylphenidate actions probably by interfering with dopamine-enhancing properties of this drug.

1. Introduction

Methylphenidate (MPH) has a long history of being an effective medication for attention-deficit/hyperactivity disorder (ADHD). The increase in stimulant prescriptions has resulted in a corresponding intensification of illicit use, particularly among college students (Advokat et al., 2008; Rabiner et al., 2009; Teter et al., 2006).

The nonmedical use of MPH has increased in the American college setting because most students report using stimulant medications in an attempt to enhance academic performance, specifically to increase concentration, organization, and the ability to stay up longer and study (Dupont et al., 2008; Godfrey, 2009). Besides, stimulants are also consumed for recreational reasons and they are often in combination with alcohol. Although stimulant therapy in childhood does not increase the risk for subsequent drug abuse in youth with ADHD (Barkley et al., 2003; Wilens et al., 2003), the recent escalation in use among adolescents and young adults has raised concern about the prevalence of stimulant diversion and misuse, and initiated debate about the ethical implications of using drugs to improve academic performance.

Recently, a systematic review was focused on MPH administration on cognitive functions in healthy humans, and the available data and the analysis performed do not allow for a conclusion to be drawn about its cognitive enhancer property (Repantis et al., 2010). In rodents, recent developmental studies demonstrating MPH effects on object recognition memory (Britton et al., 2007; Heyser et al., 2004; LeBlanc-Duchin and Taukulis, 2007) and memory for learned contextual fear associations (Britton et al., 2007) suggest that hippocampal-sensitive tasks are affected by MPH exposure during adolescence.

Similar to other psychostimulants, the dopaminergic system is one of the main targets of pharmacological action for MPH (Gatley et al.,

Abbreviations: ADHD, Attention-deficit/hyperactivity disorder; DMSO, dimethyl sulfoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MPH, Methylphenidate; SHR, Spontaneously hypertensive rats; VMAT2, Vesicular monoamine transporter 2.

* Corresponding author. Federal University of Rio Grande do Sul, Health and Basic Sciences Institute, Department of Biochemistry, Laboratory of Studies on the Purinergic System, Rua Ramiro Barcelos, 2600 anexo, Bairro Santana, Porto Alegre/RS, Brazil 90035-003. Tel.: +55 51 3308 5556; fax: +55 51 3308 5540.
E-mail address: loporciuncula@yahoo.com (L.O. Porciúncula).

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1999), albeit the participation of other neurotransmitters may also take place (Pascoli et al., 2005; Prieto-Gómez et al., 2005). Adenosine is a neuromodulator in the central nervous system (CNS), which via mainly adenosine A_1 and A_{2A} metabotropic receptors control synaptic transmission of neurotransmitters such as dopamine and glutamate (Cunha, 2001).

Adenosine A_1 receptors are expressed throughout the brain while adenosine A_{2A} are more restricted to the basal ganglia. The existence of selective heteromerization of A_{2A} and D_2 receptors and A_1 and D_1 receptors was firstly demonstrated in transfected cells followed by biochemical analysis (Agnati et al., 2003; Canals et al., 2003; Ginés et al., 2000), therefore demonstrating the existence of A_{2A}–D_2 and A_1–D_1 receptor heteromers in the brain (Ferré et al., 1997; Franco et al., 2007). The A_1–D_1 heteromeric receptor complex may therefore give the molecular basis for the well-documented antagonistic A_1–D_1 receptor/receptor interactions found in the neuronal networks of the brain (Ferré et al., 1997; Fuxe et al., 1998). The understanding of these receptor/receptor interactions has been useful for the development of novel treatments for some neuropathologies that include ADHD, Parkinson's disease, dyskinesias, schizophrenia, and drug addiction (for reviews see Ferré et al., 2008; Fuxe et al., 2007; Maggio et al., 2009).

The participation of adenosinergic system in the ADHD has been suggested in studies with spontaneously hypertensive rats (SHR) as an experimental model of ADHD. Caffeine (a non-selective adenosine receptors antagonist) and selective adenosine receptors antagonists reversed the memory impairment in this strain (Pires et al., 2009; Prediger et al., 2005).

The involvement of adenosine receptors on MPH-mediated behavioral alterations has been reported in studies where administration of caffeine induces cross tolerance and cross desensitization to MPH (Boeck et al., 2009; Jain and Holtzman, 2005). In addition, our group has reported that acute administration of MPH increases adenosine A_1 receptors density in the frontal cortex but its blockade did not blunt MPH-induced anxiolytic effect (Mioranzza et al., 2010). Apart from these studies, possible modifications in the adenosinergic system in the MPH-mediated effects are still incipient.

Considering the misuse of MPH as a cognitive enhancer, the present study was designed to assess if acute and chronic treatment MPH and an acute overdosage of MPH in adult mice could promote beneficial effects on aversive and recognition memory. Besides, the involvement of adenosine A_1 receptors was investigated in the MPH-mediated behavioral alterations.

2. Methods

2.1. Animals

Male albino CF1 mice (3–4 months old) were obtained from Stated Foundation for Health Science Research (FEPPS, Porto Alegre/RS, Brazil). All experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and to the Brazilian Society for Neuroscience and Behaviour (SBNeC) recommendations for animal care. Experimental procedures were approved by the ethical committee of Federal University of Rio Grande do Sul and all adequate measures were taken to minimize pain or discomfort. Mice were housed in standard cages (4 animals per cage) under a reversed 12/12 h–light/dark cycle with free access to food and water. The lights are turned on at 7:00 p.m. All behavioral tests were performed between 8:00 am and 5:00 p.m. Separate groups of mice were used for each behavioral task.

2.2. Drugs

A single injection of methylphenidate hydrochloride [MPH, 5 or 50 mg/kg, i.p., diluted in saline (0.9 g %, i.p) with a drop of Tween 20] or saline was administered to mice immediately after training sessions for behavioral analysis. For chronic treatment, MPH (5 mg/kg, i.p) was administered during 15 consecutive days. MPH 5 mg/kg has been widely used in behavioral studies in mice at different ages (Guerriero et al., 2006; McFadyen-Leussis et al., 2004). In order to avoid possible acute effects of the drug, the last injection was performed 12 h before mice had been submitted to behavioral tests. The adenosine A_1 receptor selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 mg/kg, i.p) (Tocris, São Paulo, Brazil) was used and prepared at the day of experiments from a stock solution diluted in DMSO (10% v/v) plus saline. This dose was chosed based on previous studies in which no behavioral alterations were observed (El Yacoubi et al., 2000; Mioranzza et al., 2010; Pires et al., 2009). Control mice received saline (0.9 g %) with a drop of Tween 20 or saline (0.9 g %). The final solution of DPCPX administered to mice contained DMSO 0.1% diluted in saline (0.9 g %).

2.3. Inhibitory avoidance task

Methylphenidate hydrochloride (MPH, 5 or 50 mg/kg, i.p) was administered to mice immediately after training session. For chronic treatment, MPH (5 mg/kg, i.p) was administered during 15 consecutive days and the last injection was performed 12 h before mice had been submitted to training session.

The inhibitory avoidance task was assessed in an apparatus that consisted of an acrylic box (50×25×25 cm) whose floor contains parallel caliber stainless-steel bars (1 mm diameter) spaced 1 cm apart. A platform (2 cm high and 4 cm×6 cm wide) was placed in the center of the box. In the training session, mice were placed on the platform and the latency to step-down on the floor with the four paws was measured with an automatic device immediately after stepping-down mice received a 0.5 mA, 2 s foot shock. After they had received the foot shock, mice were immediately placed back in their home cage. The test session was carried out 90 min after training (short-term memory) or 24 h after training (long-term memory). No foot shock was given in the test session, and step-down latencies (180 s ceiling) were taken as a measure of retention.

2.4. Object recognition task

DPCPX was administered thirty minutes prior to the training session. A single injection of methylphenidate hydrochloride (MPH 5 or 50 mg/kg, i.p) was administered to mice immediately after training sessions. For chronic treatment, MPH (5 mg/kg, i.p) was administered during 15 consecutive days. In order to avoid possible acute effects of MPH, the last injection was performed 12 h before mice had been submitted to training session. The object recognition task was performed according to previously reported (Costa et al., 2008) and following the guidelines previously recommended (Bevins and Besheer, 2006). The apparatus consisted of a painted wood small chamber with the following dimensions: 25×25 cm; (lenght×width). Mice had been acclimated in the apparatus during ten minutes twenty-four hours before training session. The training session consisted of placing a mouse in the apparatus containing two identical objects, and allowed it to explore for 10 min. The objects were positioned in two adjacent corners, 9 cm from the wall. Each mouse was always placed in the apparatus facing the wall. The test session was performed 90 min after training, and two dissimilar objects were present, the familiar (one of the objects used in the training session) and a novel one. Both objects presented similar textures, colors and sizes, but different shapes in the test session (Duplo Lego toys). The objects and the apparatus were cleaned with 10% ethanol solution between trials. Exploration was defined as directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose or forepaws. Sitting or leaning on the object without focused was not considered as exploratory behavior. For training session the index was calculated by

the ratio between the time spent on the object that will be the familiar in the test session and the total time of exploration. Considering that during training session both objects are novel, the time spent on both objects should be similar and the recognition index should be around 0.5. For calculating training index, the time spent on the object that will be the familiar in the test session was used. Recognition index for the novel object each mouse was expressed by TN / (TN + TF) ratio [TF = time spent exploring familiar object; TN = time spent exploring the novel object]. Two experienced observers blind to the drug treatment performed the behavioral analysis.

2.5. SDS-PAGE (sodium dodecyl sulfate-polyacrilamide) immunoblotting

Twenty-four hours after behavioral tests, mice were sacrificed by cervical displacement; the hippocampus and pre-frontal cortex were dissected out and immediately homogenized in 5% SDS with a protease inhibitor cocktail (Sigma, São Paulo/Brazil) and frozen at −70 °C. After defrost, the protein content was determined by Bicinchoninic acid assay using bovine serum albumin (BSA) as standard (Pierce, São Paulo/Brazil). Samples extracts were diluted to a final protein concentration of 2 μg/μL in SDS-PAGE buffer. Forty micrograms of the samples and prestained molecular weight standards (Bio-Rad, São Paulo/Brazil) were separated by SDS-PAGE (12% with 4% concentrating gel). After electro-transfer, the membranes were blocked with Tris-buffered saline 0.1% Tween-20 (TBS-T) containing 3% BSA for 1 h. The membranes were then incubated for 24 h at 4 °C with rabbit anti-adenosine A_1 receptor antibody (1:1000; Affinity Bioreagents, U.S.A). After primary antibodies incubation, membranes were washed and incubated with horseradish-peroxidase conjugated secondary antibodies for 2 h at room temperature and developed with chemiluminescence ECL kit (Amersham, São Paulo/Brazil). The autoradiographic films were scanned and densitometric analyses were performed using public domain NIH Image Program (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). The control of protein loading was carried out with Ponceau S. stain. Membranes with Ponceau S. were scanned at 37 kDa and the values used to obtain adenosine A_1 receptor density/Ponceau S. density ratio. No differences were found in the amount of protein loaded (data not shown).

2.6. Statistical analysis

For the inhibitory avoidance task some animals reached the ceiling of 180 s and the data distribution did not follow Gaussian curve. Thus, the step-down latencies were expressed as medians (interquartile ranges) and non-parametric analysis was performed by using Wilcoxon test for differences between training and test latencies of the same group. Kruskal–Wallis followed by Dunn's Multiple comparison test was used to compare treatments. For object recognition test, two-way ANOVA (treatment × trials) as repeated measures (as independent variables) was performed. For immunoblotting, data were analyzed by using Student's t-test between groups. Graphpad Prism 5 and SPSS were the softwares used and significant differences were considered when $P<0.05$.

3. Results

3.1. Acute administration of methylphenidate in the inhibitory avoidance task performance

The administration of MPH 5 mg/kg immediately after training did not modify the latencies between training and test compared to saline-treated mice when test session was performed 90 min (Fig. 1A) as well as 24 h after training (Fig. 1B). However, latencies were statistically different between mice treated with an overdosage of

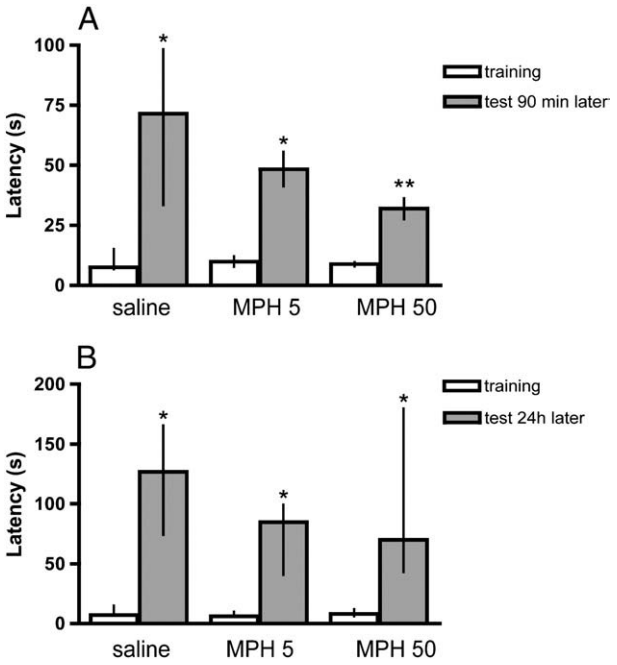

Fig. 1. Acute treatment with methylphenidate (MPH) on the performance in the inhibitory avoidance task. Adult mice receive a single injection of MPH (5 or 50 mg/kg, i.p) or vehicle immediately after training session. A — Test session performed 90 min after training (short-term memory). Results are median and interquartile ranges of step-down latency in seconds (s) from 11 to 12 mice per group. B — Test session performed 24 h after training (long-term memory). Results are median and interquartile ranges of step-down latency in seconds (s) from 11 to 12 mice per group. $^{**}P<0.05$; different from latencies obtained in the test session for saline-treated mice (Kruskal–Wallis followed by Dunn's post hoc test). $^{*}P<0.05$; differences between latencies from training and test sessions within group (Wilcoxon test).

MPH 50 mg/kg and saline only for test session performed 90 min (Fig. 1A) but not for 24 h after training (Fig. 1B).

3.2. Chronic administration of methylphenidate in the inhibitory avoidance task performance

Mice submitted to the chronic treatment with MPH 5 mg/kg were also evaluated in the inhibitory avoidance task. The latencies of training and test session between saline and MPH-treated mice were not statistically significant for test session performed 90 min after training as well as for 24 h (data not shown).

3.3. Acute treatment of methylphenidate in the object recognition task

The influence of chronic and acute treatment with MPH 5 mg/kg and an acute overdosage of 50 mg/kg was investigated in the novel object recognition task that consists on a non-aversive task. Two-way ANOVA analysis for the total exploration time in both objects for acute administration of MPH 5 mg/kg revealed a significant effect of trials (as repeated measures) [$F(1,41)=46.88$; $P<0.001$] but no significant interaction between trials and treatment (Fig. 2A). As a normal behavior, mice spent less time on the familiar object in the test session when comparing to training. Two-way ANOVA treatment × trials (as repeated measures) revealed only a significant main effect of trials [$F(1,41)=77.59$; $P<0.001$] (Fig. 2B). For the object recognition index, two-way ANOVA revealed a significant main effect of trials and significant interaction [$F(1,41)=4.24$; $P=0.0405$] (Fig. 2C). As observed, acute administration of MPH 5 mg/kg caused an increase in the object recognition index.

The acute administration of MPH 50 mg/kg did not affect the total exploration time in both objects since two-way ANOVA analysis revealed only significant effect of trials [$F(1,20)=88.87$; $= P<0.001$]

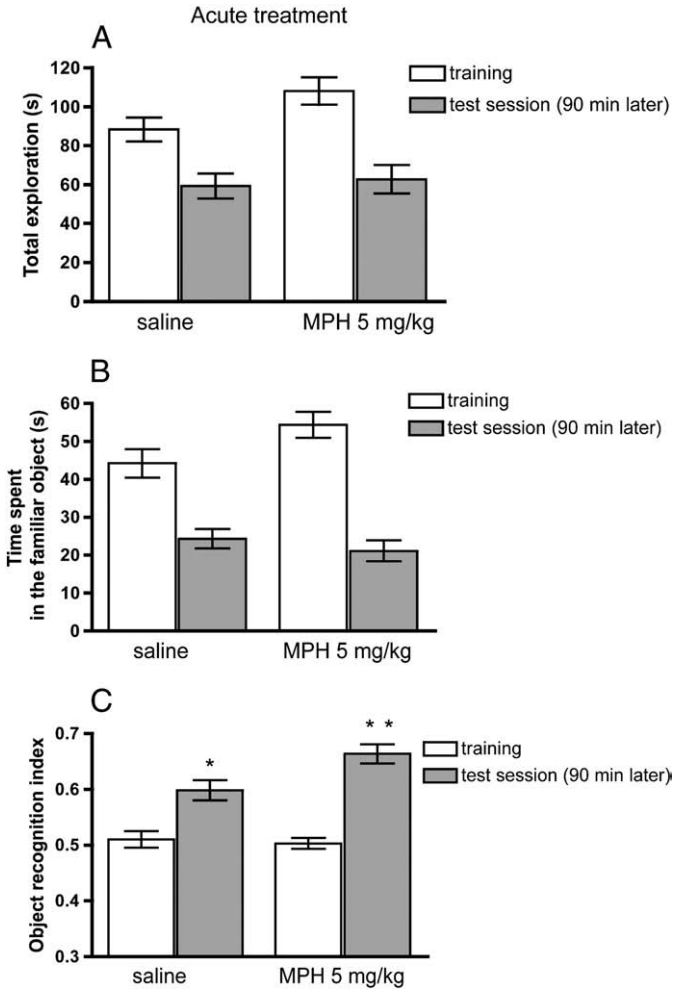

Fig. 2. Acute treatment with methylphenidate (MPH) on the performance in the object recognition task. Adult mice received a single injection of MPH (5 mg/kg, i.p) or vehicle immediately after training session. A – Total time spent in both objects during training and test session performed 90 min after training. Results are mean ± S.E.M from 21 mice per group. B — Time spent in the familiar object in both sessions (training and test session) Results are mean ± S.E.M from 21 mice per group. C — Recognition index obtained from training and test session. Results are mean ± S.E.M from 21 per group mice. *$P<0.05$; differences within group (training and test session) (two-way ANOVA). **$P<0.05$; differences within groups and from saline-treated mice recognition index (two-way ANOVA).

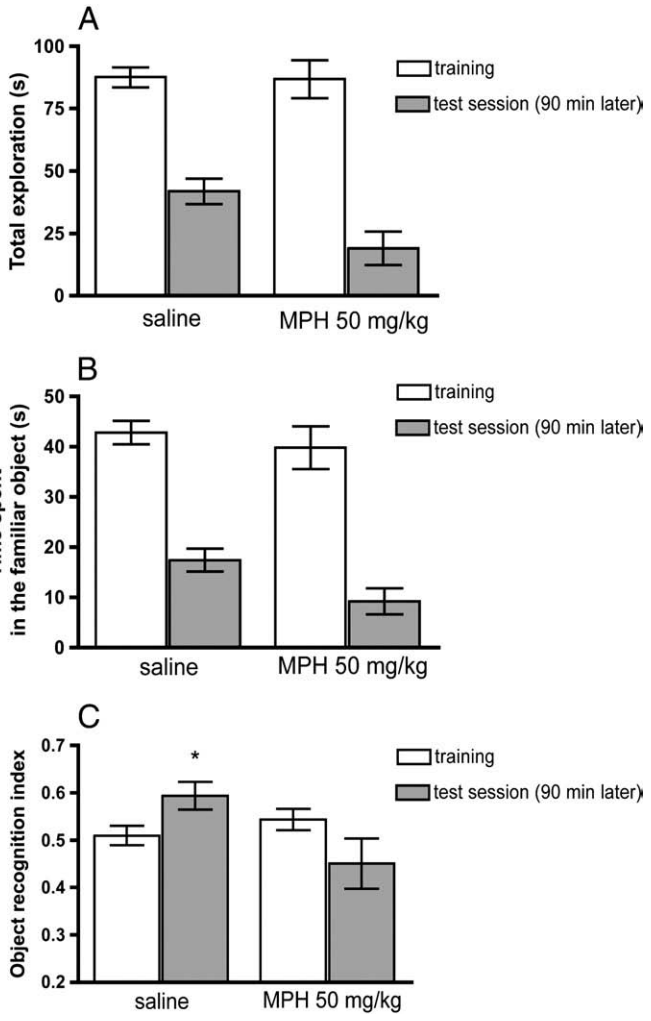

Fig. 3. Acute treatment with methylphenidate (MPH) on the performance in the object recognition task. Adult mice received a single injection of MPH (50 mg/kg, i.p) or vehicle immediately after training session. A — Total time spent in both objects during training and test session performed 90 min after training. Results are mean ± S.E.M from 10 to 12 mice per group. B — Time spent in the familiar object in both sessions (training and test session) Results are mean ± S.E.M from 10 to 12 mice per group. C — Recognition index obtained from training and test session. Results are mean ± S.E. M from 10 to 12 mice per group. *$P<0.05$; different from MPH-treated mice and training session within group (two-way ANOVA).

(Fig. 3A). However, the interaction was almost significant [F (1,20) = 3.35; $P<0.09$]. The same result was observed for the time spent in the familiar object, since two-way ANOVA revealed only significant effect of trials [F (1,20) = 97.88; = $P<0.001$] (Fig. 3B). For the object recognition index, two-way ANOVA revealed a significant interaction [F (1,20) = 7.07; $P=0.0151$] but no significant effect of trials (Fig. 3C). Thus, the overdosage of MPH 50 mg/kg decreased object recognition index in the test session.

3.4. Chronic treatment with methylphenidate in the object recognition task

The chronic treatment with MPH 5 mg/kg was also evaluated on recognition memory. Analysis of time spent on the familiar object revealed a significant effect of trials [F (1,16) = 69.95; = $P<0.001$] (Fig. 4A). Likewise, two-way ANOVA analysis for recognition index also revealed only a significant effect of trials [F (1,16) = 5.84; = $P=0.0279$] (Fig. 4B).

3.5. The effect of blockade of adenosine A_1 receptors on MPH-induced recognition memory impairment

The involvement of adenosine A_1 receptors in the recognition memory impairment by MPH 50 mg/kg was investigated with the selective adenosine A_1 receptor antagonist DPCPX (1 mg/kg, i.p) administered 30 min before training session. MPH (50 mg/kg, i.p) was administered immediately after training. According to previous data, MPH 50 mg/kg did not modify the time spent in the familiar object and administration of DPCPX alone did not cause any effect (data not shown). Two-way ANOVA analysis of the recognition index revealed a significant effect of trials [F (1,48) = 33.42; $P<0.001$] and interaction [F (1,48) = 3.47; $P=0.0206$]. Consequently, pre administration of DPCPX prevented the decrease on the recognition index caused by post training administration of MPH 50 mg/kg (Fig. 5).

3.6. Chronic administration of methylphenidate on adenosine A_1 receptors immunocontent

Immunoblotting analysis was carried out in the frontal cortex and hippocampus homogenates from mice treated chronically with saline

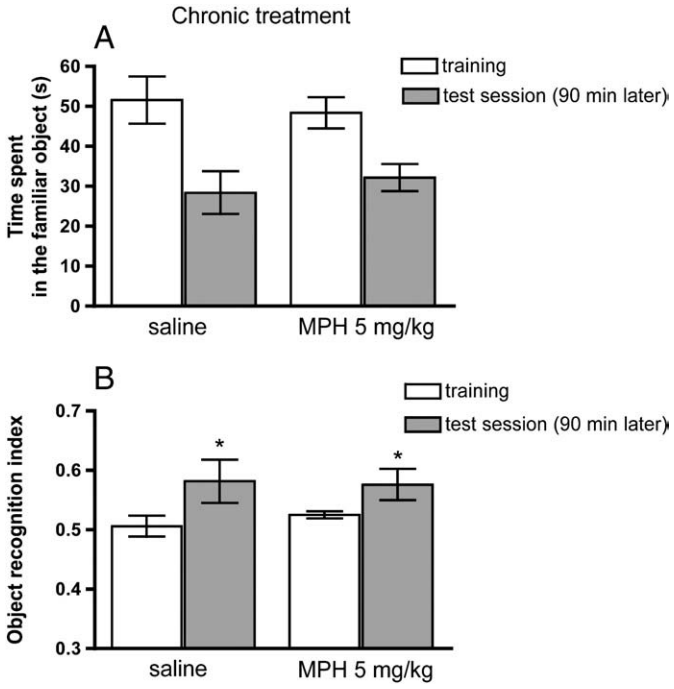

Fig. 4. Chronic treatment with methylphenidate (MPH) on the performance in the object recognition task. Adult mice received a single injection of MPH 5 mg/kg or vehicle during 15 consecutive days. The last dose of MPH was administered 12 h prior to training session. A — Time spent in the familiar object in both sessions (training and test session). Results are mean ± S.E.M from 9 mice per group. B — Recognition index obtained for training and test session. Results are mean ± S.E.M from 9 mice per group. *$P<0.05$; different from training session within group (two-way ANOVA).

or MPH (5 mg/kg) ($t=0.53$; $P>0.05$). Chronic treatment with MPH 5 mg/kg decreased the density of adenosine A_1 receptor only in the hippocampus (15%) compared to saline-treated mice (Fig. 6) ($t=2.855$; $P=0.03$).

4. Discussion

In this study, the acute and chronic administration as well as an overdosage of methylphenidate caused behavioral alterations in adult animals. Here, acute and chronic administration of MPH 5 mg/kg and an acute overdosage to model misuse presented distinct effects according to the task used for evaluating memory. Besides, the role of adenosine A_1 receptors was involved in the effects of MPH on recognition memory.

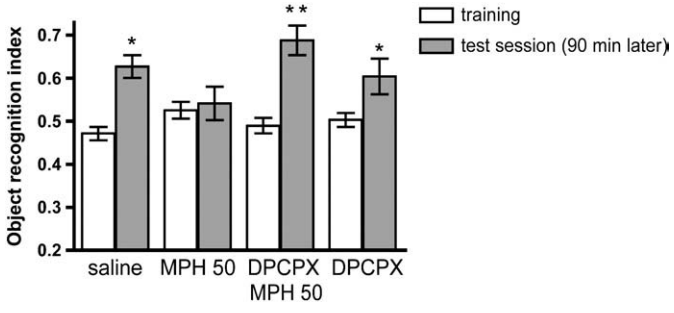

Fig. 5. Blockade of adenosine A_1 receptors prevents MPH-induced impairment on memory recognition. DPCPX (1 mg/kg, i.p) was administered 30 min before training session. MPH (50 mg/kg, i.p) was administered immediately after training. Recognition index obtained from training and test session performed 90 min later. Results are mean ± S.E.M from 12 to 15 mice per group. *$P<0.05$; different from training session within group (two-way ANOVA). **$P<0.05$; different from recognition index in the test session obtained for MPH-treated mice (two-way ANOVA).

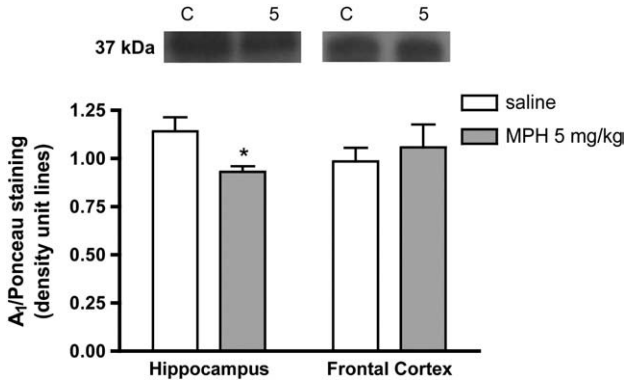

Fig. 6. Immunoblotting analysis of the adenosine A_1 receptor density in the frontal cortex and hippocampus from adult mice. Mice chronically treated either with vehicle or methylphenidate (MPH, 5 mg/kg, i.p. 15 days). Data are mean ± S.E.M of density unit lines obtained for adenosine A_1 receptor/Ponceau staining ($n=5$ mice/group for hippocampus), ($n=6$ mice/group for frontal cortex). At the top of figure are representative bands for adenosine A_1 receptor at 37 kDa (saline-treated mice; 5-methylphenidate-treated mice). *$P<0.05$, means significant difference between MPH- and saline-treated mice (Student's t-test).

Recognition memory was assessed in the novel object recognition task, which deals with the natural ability of animals to explore novelties. In this study, chronic administration of methylphenidate in a dose used in behavioral studies with juvenile mice did not cause any effect on both types of memory in adult mice whereas acute administration improved recognition memory. One explanation for this improvement could be attributed to both dopamine and norepinephrine-enhancing properties of the drug. Stimulant drugs, such as methylphenidate, raise extracellular dopamine levels supposedly by blockade of the dopamine transporter. This mechanism prevents reuptake of dopamine into the neuron, which results in higher extracellular dopamine levels (Madras et al., 2005; Volz et al., 2005). Additionally, acute administration of methylphenidate redistributes vesicular monoamine transporter 2 (VMAT2) protein from membrane associated vesicles fraction to cytoplasmic vesicles, which results in an increase in dopamine content in both fractions (Fleckenstein et al., 2009; Volz et al., 2008). Recent experimental evidence have demonstrated that dopamine is essential for memory consolidation in a variety of behavioral tasks, such as the hidden version of the water maze, the object–place association, and the one-trial inhibitory avoidance tasks (Dalley et al., 2005; Ferretti et al., 2005; Setlow and McGaugh, 2000). Besides, dopamine signaling is crucial for memory persistence as evidenced in the one-trial inhibitory avoidance task (Rossato et al., 2009).

Interestingly, the acute overdosage impaired both types of memory but the magnitude of memory impairment was dependent on the type of memory assessed, since recognition memory was fully impaired, while in the inhibitory avoidance task memory was partially impaired. In fact, in the inhibitory avoidance task mice treated with the overdosage of methylphenidate presented differences between training and test latencies indicating that they recalled the aversive stimuli (foot shock), but not at the same level of saline-treated mice. Therefore, MPH disrupts recognition memory and worsens the performance in the inhibitory avoidance task when short-term memory was assessed. Aversive and recognition memories share the functioning of some brain areas such as the hippocampus, ventral tegmental area, *striatum* and pre-frontal cortex. Differently from object recognition task, the amygdala is highly involved in memories with emotional component such as those assessed in the inhibitory avoidance task. The differences found for the effects of methylphenidate according to the type of memory assessed could be due to the fact that this drug administered intraperitoneally increases the extracellular dopamine levels in the striatum, nucleus accumbens and pre-frontal cortex, with no clear evidences of increase in the

amygdala (Bymaster et al., 2002; Koda et al., 2010). Recently, local administration of methylphenidate in the lateral amygdala enhanced cue-reward learning through dopamine D1 receptor-dependent mechanisms and suppressed task-irrelevant behavior through D2 receptor-dependent mechanisms. These findings suggest distinct roles for dopamine receptor subtypes in mediating methylphenidate-induced enhancements of neural transmission and learning performance (Tye et al., 2010). It is likely that overactivation of dopamine and norepinephrine may contribute to the disruption of learning and memory as well (for review see Arnsten, 2001).

The long-term memory was preserved by the overdosage of MPH probably due to the fast clearance of this drug, since pharmacokinetic analysis studies have shown that the half-life of MPH in rodents after intraperitoneal administration is estimated to be around 1 h (Thai et al., 1999). In fact, when long-term memory was assessed mice had received the injection at least 36 h prior to test session.

Chronic administration of MPH 5 mg/kg in adult mice did not cause any effect on both types of memory. Conflicting results with chronic administration of MPH in different dosing regimen and age have been reported. For example, the administration of similar regular dose in previous studies has shown to impair recognition memory, but the age (periadolescent rats) at the beginning of treatment differs from our study (Heyser et al., 2004; LeBlanc-Duchin and Taukulis, 2007). However, adult rats treated with MPH 5 and 10 mg/kg showed impairment on recognition memory but this effect was evident only 14 days later (LeBlanc-Duchin and Taukulis, 2009). In a recent study, rats presented lower performance in the Water maze test after they had received MPH (2 mg/kg) from the 15th to the 45th day of age (Scherer et al., 2010). However, male and female rats treated with 3 mg/kg of MPH from the 22nd to the 39th day of age presented an improvement on the radial arm maze performance after seven days of treatment (Zhu et al., 2007). The schedule of administration, differences between animal species (rats versus mice), age at the beginning of treatments and strains may take part of the discrepancies found between behavioral findings. Prominently, animals treated with MPH presented distinct behavior depending on the circadian cycle. Similar to our findings, in the one-trial inhibitory avoidance task chronic treatment of adult rats during 28 days with MPH (2 mg/kg) did not cause any effect, but in a multiple trial protocol long-term memory was impaired when animals were tested at night (Gomes et al., 2010). Interestingly, in the same study the long-term memory in young rats was impaired in the one-trial as well as in the multiple trial of the inhibitory avoidance task. As ontogenetic differences are considered one of the main factors responsible for distinct psychopharmacological sensitivity in a variety of species, adolescent rodents seem to be more sensitive than their adult counterparts to effects caused by psychostimulants (for review see Spear, 2000).

Alterations in the locomotor activity of mice could interfere in the performance of the object recognition task, but previous results from our group revealed that the same dose and schedule of administration did not alter locomotor activity (Mioranzza et al., 2010). However, a decrease on novelty seeking or anhedonia caused by an overdosage of methylphenidate could not be discarded as well as the possibility that methylphenidate affected motivation, attention, sensorimotor function, or memory retrieval. Indeed, previous study showed that adult rats exposed to repeated doses of methylphenidate during their juvenile were less responsive with respect to motor activation exhibited by animals when first exposed to a novel environment (Bolaños et al., 2003).

In our previous study, anxiolytic-like effect caused by MPH was not blunted by the blockade of adenosine A_1 receptors and there was an up-regulation in the frontal cortex of this receptor by an overdosage of methylphenidate (Mioranzza et al., 2010). Thus, the up-regulation of this receptor could be involved in the impairment of recognition memory observed in this study. Although chronic administration of MPH was devoid of effect on memory, the immunocontent of adenosine A_1 receptors was decreased in the hippocampus. Overall, it remains to be determined if acute treatment with MPH could increase brain adenosine levels and a continuous administration could trigger the desensitization of adenosine A_1 receptors. It is interesting to note that caffeine, another well-known psychostimulant and a non-selective adenosine antagonist, also up-regulates adenosine A_1 receptors (Svenningsson et al., 1999).

The stimulation of adenosine receptors counteracts the behavioral effects of dopamine receptor stimulation (Cao et al., 2007; Ferré et al., 1997). Likewise, adenosine receptor agonists counteract whereas adenosine receptor antagonists potentiate pharmacological effects of psychostimulants like cocaine and amphetamines (Poleszak and Malec, 2003; Popoli et al., 1994; Rimondini et al., 1998). Particularly, behavioral alterations caused by other psychostimulants acting on dopaminergic system were related to adenosine A_1 receptors (Kuzmin et al., 1999; Poleszak and Malec, 2003). Our results are in agreement with recent report where DPCPX did not promote any effect on recognition memory, but its administration was effective in ameliorating the impairment of novel object recognition in spontaneously hypertensive rats (SHR), used as a model of ADHD (Pires et al., 2009).

In human adults with and without ADHD, there are only few studies that have examined the effects of methylphenidate on cognitive functions. In adult ADHD some studies found methylphenidate to improve working memory (Kurscheidt et al., 2008; Mehta et al., 2000; Turner et al., 2005). In healthy subjects, methylphenidate enhanced performance in a test of spatial working memory and planning whereas impaired attention and fluency tests (Elliott et al., 1997). In our study, improvement on recognition memory was observed with a single dose of methylphenidate acutely administered while chronic administration did not show any effect. Therefore, our findings suggest that chronic use of MPH as a cognitive enhancer did not offer evident advantages for healthy animals.

5. Conclusion

As a cognitive enhancer, our data showed that methylphenidate acutely administered in a single dose promoted improvement on recognition memory. However, the overdosage caused disturbances in aversive as well as non-aversive memories at least when mice were under influence of this drug, suggesting that misuse of MPH may impair important cognitive functions. The chronic administration did not promote neither detrimental nor beneficial effects on memory. Importantly, methylphenidate-induced impairment on recognition memory involved adenosine A_1 receptors suggesting that this receptor plays a role in the mnemonic deficits caused by MPH. Since adenosine is a neuromodulator that controls the dopaminergic neurotransmission, which is one of the main pharmacological targets of MPH, it is important to detail the participation of adenosinergic system in the ADHD and methylphenidate-mediated actions in the CNS.

Disclosure of conflicts of interest

The authors have no disclosures to declare.

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